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Silicon alleviates PEG-induced osmotic stress in finger millet by regulating membrane damage, osmolytes, and antioxidant defense

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Abstract

Drought restricts plant growth and productivity. Silicon has beneficial effects on imparting drought tolerance in plants. Present work was intended to evaluate the effect of Si on polyethylene glycol-6000 (PEG) induced osmotic stress in local landraces of finger millet. The seeds of stress-tolerant and stress-sensitive landraces of finger millet were treated with distilled water, 15% PEG, and PEG+Si (5-25 ppm). The ameliorative effect of Si was evaluated in terms of percentage seed germination, seedling growth, accumulation of osmolyte and activity of antioxidative enzymes. PEG-induced osmotic stress reduced seed germination, seedling growth, and augmented osmolyte accumulation. It also elevated the levels of antioxidant enzymes. The exogenous supplementation of silicon significantly improved seed germination as well as early seeding growth. Positive effects of Si were reflected in decline in malondialdehyde (MDA) content and improved glycine betaine content and antioxidant enzymes in PEG-induced stress tolerant as well as susceptible landraces. The Si-induced ameliorated effects on all the parameters studied were more pronounced in the stress-tolerant landrace (FM/ST/01) than the stress-sensitive landrace (FM/RT/01). These results clearly indicate advantageous effects of Si in relieving PEG-induced stress during seed germination and early seeding growth and suggest a possibility of better stand establishment by application of silicon containing fertilizer during seed sowing.

Keywords: antioxidative enzymes; finger millet; osmolytes; osmotic stress; PEG; silicon

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Introduction

Silicon (Si), the second most abundant component in the earth's crust (Epstein, 1999), is present in substantial quantities in all plants (Ma *et al*., 2006). Accumulation of Si fluctuates from 0.1% to 10% in whole plants (Epstein, 1994), which is ascribed to the discrete competence of roots to uptake it (Takahashi *et al*., 1990). It acts as a physical or mechanical obstacle in plants, and is vigorously involved in many metabolic and physiological processes (Saud *et al*., 2014). Many plants with lower Si content show reduced growth and yield in addition to higher vulnerability to environmental stresses (Datnoff *et al*., 2001). Supportive effects of Si against environmental stresses such as salinity, drought, temperature, heavy metal and UV radiation have also been reported (Lekklar *et al*., 2019).

Beneficial effects of silicon include mitigation of drought stress through various physiological processes (Mundada *et al*., 2021). It has efficiently lessened the injury caused to the photosynthetic pigments, stomatal frequency, thickness of leaf blades (Rezende *et al*., 2018) and has affected the rate of transpiration in plants by influencing stomatal movement (Gao *et al*., 2006). Supplementation of silicon had resulted in the development of a double layer of Si-cuticle on leaf epidermal cells, which in turn improved the leaf water potential (Matoh *et al*., 1991). Hattori *et al*. (2005) have demonstrated the essential role of Si in root growth and movement of water from the rhizosphere of *Sorghum*. The role of silicon in efficient use of water in wheat has also been reported (Gong *et al*., 2003).

Millets are the fundamental source of calories and proteins in the tropical regions of Asia and Africa. Finger millet, *Eleusine coracana* (L.) Gaertn. (Poaceae) occupies the fourth position among millets (Antony Ceasar *et al*., 2018). It is predominantly cultivated in 25 countries from African and Asian continents and represents about 12% of the common millet cultivation area. Worldwide, around 4.5 million tons of finger millet are produced every year. Of this, Africa produce 2.5 million tons and India produces 1.2 million tons annually (Antony Ceasar *et al*., 2018). Although finger millet is regarded as an under-utilized cereal crop, it is a vital food resource for rural populations. In India, it is cultivated on more than 1.19 million ha of land with average production of approximately 1600 kg per ha (Sakamma *et al*., 2018). Nutritionally, it is a rich source of calcium, dietary fibers, phenolic compounds, and also hold various pharmaceutical and pharmacological activities (Devi *et al*., 2014).

Finger millet can withstand severe osmotic stress and exhibits notable retrieval on mitigation of stress (Uma *et al*., 1995; Govind *et al*., 2009). Conventional breeding techniques as well as modern crop improvement technologies are being used to develop high-yielding varieties such as GPU28 and Indaf 9 (Nagaraja *et al*., 2008; Shet *et al*., 2009; Nandini *et al*., 2010). Finger millet is vulnerable to environmental stresses, particularly salinity and drought-induced osmotic stress, during seed germination and early stages of seedling development (Mundada *et al*., 2020). Therefore, developing stress-tolerant finger millet varieties to cope up with oxidative stress during these stages is imperative prerequisite. Many investigations have reported involvement of Si in the regulation of plant water relations and physicochemical changes under stressful conditions in various crops like rice, wheat, jowar, maize, soybean etc. (Saud *et al*., 2014). However, the effects of exogenous supplementation of Si on the performance of finger millet under drought stress have not been studies so far. Therefore, the present investigation was designed to study the ameliorative effects of exogenous Si supplementation in drought-tolerant and sensitive landraces of finger millet exposed to PEG-6000-induced osmotic stress.

Materials and Methods

Plant material

Seeds of local finger millet landraces were collected from the farmers from different regions in western Maharashtra (Mundada *et al*., 2019). These landraces were screened for stress tolerance capacity by using PEG-6000 induced osmotic stress as described earlier (Mundada *et al*., 2020). The stress-tolerant (FM/ST/01) and sensitive (FM/RT/01) landraces were selected to study the ameliorative effect of exogenous Si supplementation.

Si treatment and culture conditions

Seeds of stress-tolerant (FM/ST/01) and sensitive (FM/RT/01) landraces were thoroughly washed under running tap water. Seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for 2 min, followed by three times washing with ample volume of sterilized distilled water to remove the traces of $HgCl_2$. Randomly selected fifty healthy seeds were allocated to each treatment. Seeds were layered on double layered germination paper in Petri dishes. The paper towel was initially moistened with 10 ml of distilled water (control), 15% PEG-6000 solution (induction of osmotic stress), and PEG + Si. The Si supplementation (aqueous H_4SiO_4) was used in the range from 5 to 25 ppm (designated as Si 1 to Si 5) with the interval of 5 ppm. To avoid desiccation of germination paper, 2 ml of initial solution was added to the respective Petri dish every day or if required. Petri dishes were incubated in dark at room temperature. All the observations were taken on the 14^{th} day after sowing.

Growth analysis

The seeds showing radicle growth of at least 0.5 mm were considered as germinated seed. Seed germination counts were taken daily up to 14 days. Final germination percentage was calculated on 14^{th} days after sowing. Seedling growth was measured in terms of shoot length, root length, and root:shoot ratio. The biomass of seedlings was measured as fresh weight, dry weight, and percentage moisture content calculated as [(Fresh weight – Dry weight)/Fresh weight] × 100.

Biochemical analysis

Membrane integrity was measured in terms of levels of lipid peroxidation [malondialdehyde (MDA) content] following Heath and Packer (1968). Among the quaternary ammonium compounds, glycine betaine content was estimated following Grieve and Grattan (1983). Osmolyte accumulation (proline) was assessed as per Bates *et al*. (1973). Total soluble sugars (TSS) were estimated using the method described by Watanabe *et al*. (2000).

Antioxidative enzyme assays

Crude enzyme extract was prepared from 500 mg fresh seedling biomass. Samples were ground in 5 ml of 50 mM ice cold sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% PVP, followed by centrifugation at 10,000 rpm for 20 min at 4 °C. The supernatant was used as the source of crude antioxidant enzymes. The superoxide dismutase (SOD) activity was estimated as per method described by Beyer and Fridovich (1987). The assay mixture (1 ml) comprised phosphate buffer (50 mM; pH 7.0), EDTA (0.1 mM), methionine (14.3 mM), NBT (82.5 µM), and freshly prepared riboflavin (2.2 µM). The reaction was initiated by addition 25 µl of crude enzyme extract and test tubes were incubated under the fluorescent tube lights for 30 min. The reaction was terminated by switching off the light source. The assay mixture without enzyme extract served as light-control, and the assay mixture with 25 µl enzyme extract incubated in the dark was considered as dark-blank. The quantification of NBT reduction was based on change in absorbance at 560 nm (UV 1800, Shimadzu, Japan). The specific enzyme activity was expressed as µKat mg^{-1} protein.

The activity of catalase (CAT) was assayed as per the method of Cakmak and Marschner (1992). Assay mixture (1 ml) consisted of H_2O_2 (15 mM) as a substrate and phosphate buffer (50 mM, pH 7.0). The reaction was initiated with the addition of 50 µl crude enzyme, and the activity was estimated from the decrease in absorbance at 240 nm (ε = 36 mM^{-1} cm^{-1}) for 2 min. The specific enzyme activity was stated as µKat mg^{-1} protein.

Ascorbate peroxidase (APX) activity was estimated as per the method described by Nakano and Asada (1981). The assay mixture comprised phosphate buffer (50 mM, pH 7.0), ascorbic acid (0.5 mM) and H_2O_2 (0.1 mM). The reaction was initiated with the addition of 50 µl of crude enzyme. The decrease in absorbance due to oxidation of ascorbate was recorded at 290 nm (ε = 2.8 mM^{-1} cm^{-1}) for 1 min. The specific enzyme activity was expressed as µKat mg^{-1} protein.

The activity of guaiacol peroxidase (GPX) was assayed by following Hemeda and Klein (1990). The assay mixture (1 ml) comprised phosphate buffer (50 mM, pH 7.0), guaiacol and crude enzyme (10 µl). The assay was initiated by addition of 200 mM H_2O_2, and an increment in absorbance was recorded at 470 nm (ε = 26.6 mM^{-1} cm^{-1}). Enzyme activity was stated as µKat mg^{-1} protein.

Statistical analysis

Completely randomized design (CRD) was followed to set the experiments. Each treatment had three replicates and all the experiments were repeated thrice. The results are stated as the mean±standard error (SE). The results were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at p=0.05 to detect significant differences among means by using statistical software IBM SPSS.

Results

Percentage germination of seeds, length of root, shoot; and root/shoot ratio were used to evaluate the influence of Si on the growth of finger millet seedlings exposed to PEG induced osmotic stress. Osmotic stress significantly affected the germination percentage by up to 8.47% (FM/ST/01) and 60.65% (FM/RT/01) as compared to their respective control (Table 1).

Table 1. Effect of Si on germination percentage, root length and shoot length of local finger millet cultivars under PEG-6000 induced osmotic stress

Name of sample	Treatments	Germination percentage (%)	Root length (cm)	Shoot length (cm)	R/S Ratio
FM/RT/01	DW	81.33±2.4^{a}	3.28±0.30^{a}	2.17±0.10^{ab}	1.51
	PEG	32.00±3.5^{d}	1.94±0.10^{b}	1.83±0.09^{cd}	1.07
	PEG + Si 1	62.67±2.9^{c}	3.44±0.34^{a}	2.20±0.12^{ab}	1.56
	PEG + Si 2	74.67±2.9^{ab}	3.26±0.16^{a}	2.45±0.06^{a}	1.33
	PEG + Si 3	67.33±4.7^{bc}	2.79±0.40^{ab}	2.02±0.10^{bc}	1.40
	PEG + Si 4	64.67±4.1^{bc}	2.73±0.36^{ab}	1.82±0.10^{cd}	1.53
	PEG + Si 5	63.33±3.5^{bc}	2.58±0.38^{ab}	1.66±0.12^{d}	1.54
FM/ST/01	DW	86.67±2.9^{ab}	3.08±0.76^{a}	2.96±0.05^{a}	2.22
	PEG	79.33±3.5^{b}	1.90±0.85^{c}	2.63±0.05^{bc}	1.35
	PEG + Si 1	82.67±4.1^{ab}	3.15±1.11^{b}	2.55±0.07^{bc}	1.99
	PEG + Si 2	94.67±1.8^{a}	3.21±1.06^{a}	2.86±0.10^{ab}	2.34
	PEG + Si 3	87.33±4.7^{ab}	2.59±0.84^{a}	2.47±0.14^{c}	2.54
	PEG + Si 4	86.67±4.7^{ab}	2.54±0.74^{a}	2.43±0.12^{c}	2.55
	PEG + Si 5	86.00±4.2^{ab}	2.30±0.72^{a}	2.35±0.12^{c}	2.58

Values represent mean±SE. Mean followed by different letters within columns are significantly different at P = 0.05 as per Duncan's Multiple Range Test (DMRT). DMRT was applied to each landrace separately. Si: Silicon; FM/RT/01: Osmotic stress sensitive landrace; FM/ST/01: Osmotic stress tolerant landrace; DW: Distilled water; PEG: Polyethylene glycol (15%); Si 1: Si 5 ppm; Si 2: Si 10 ppm; Si 3: Si 15 ppm; Si 4: Si 20 ppm and Si 5: Si 25 ppm.

Incorporation of Si (10 ppm) enhanced the seed germination percentage by 19.34% and 133.34% in osmotic stress-tolerant (FM/ST/01) and sensitive (FM/RT/01) landraces respectively, as compared to seed germination under the PEG-6000 induced osmotic stress (Table 1).

Incorporation of 10 ppm Si reduced the adverse effect of osmotic stress on the length of root, shoot and their ratios in the seedlings of both landraces. Improvement in seedling growth was higher in osmotic stress-tolerant (FM/ST/01) landrace as compared to stress-sensitive (FM/RT/01) landrace. Decrease in the total seedling dry weight due to osmotic stress was not significant. Though significant increase in dry biomass was observed in seedlings simultaneously exposed to osmotic stress and silicon (PEG + Si 2), it was still less than that observed in control of tolerant landrace (Table 2).

Table 2. Effect of Si on fresh weight, dry weight and moisture content in seedlings of local finger millet landraces under PEG-6000 induced osmotic stress

Name of sample	Silicon concentration	Fresh weight (gm)	Dry weight (gm)	Moisture content (%)
FM/RT/01	DW (Control)	0.95 ± 0.02^{a}	0.07 ± 0.01^{b}	92.65
	PEG (15 %)	0.87 ± 0.03^{cd}	0.08 ± 0.01^{b}	90.79
	PEG + Si 1	0.94 ± 0.03^{ab}	0.08 ± 0.01^{b}	91.46
	PEG + Si 2	0.99 ± 0.04^{a}	0.11 ± 0.02^{a}	88.93
	PEG + Si 3	0.97 ± 0.04^{a}	0.09 ± 0.02^{ab}	90.44
	PEG + Si 4	0.89 ± 0.04^{bc}	0.08 ± 0.01^{b}	91.00
	PEG + Si 5	0.82 ± 0.04^{d}	0.07 ± 0.01^{b}	91.24
FM/ST/01	DW (Control)	1.51 ± 0.08^{b}	0.18 ± 0.03^{a}	87.97
	PEG (15 %)	1.17 ± 0.07^{c}	0.14 ± 0.04^{a}	87.61
	PEG + Si 1	1.29 ± 0.04^{c}	0.15 ± 0.03^{a}	88.38
	PEG + Si 2	1.81 ± 0.08^{a}	0.18 ± 0.03^{a}	90.29
	PEG + Si 3	1.35 ± 0.14^{bc}	0.16 ± 0.04^{a}	88.46
	PEG + Si 4	1.34 ± 0.11^{bc}	0.15 ± 0.03^{a}	88.88
	PEG + Si 5	1.24 ± 0.12^{c}	0.14 ± 0.03^{a}	88.46

Values represent mean±SE. Mean followed by different letters within columns are significantly different at $P = 0.05$ as per Duncan's Multiple Range Test (DMRT). DMRT was applied to each landrace separately. Si: Silicon; FM/RT/01: Osmotic stress sensitive landrace; FM/ST/01: Osmotic stress tolerant landrace; DW: Distilled water; PEG: Polyethylene glycol (15%); Si 1: Si 5 ppm; Si 2: Si 10 ppm; Si 3: Si 15 ppm; Si 4: Si 20 ppm and Si 5: Si 25 ppm.

Membrane damage was evaluated in terms of MDA accumulation in the seedlings of finger millet. More accumulation of MDA was observed in osmotically stressed seedlings of both the landraces as compared to their respective control. Supplementation of Si mitigated MDA accumulation. Increase in the MDA level due to osmotic stress was more pronounced in osmotic stress-sensitive (FM/RT/01) landrace than tolerant (FM/ST/01) landrace (Figure 1).

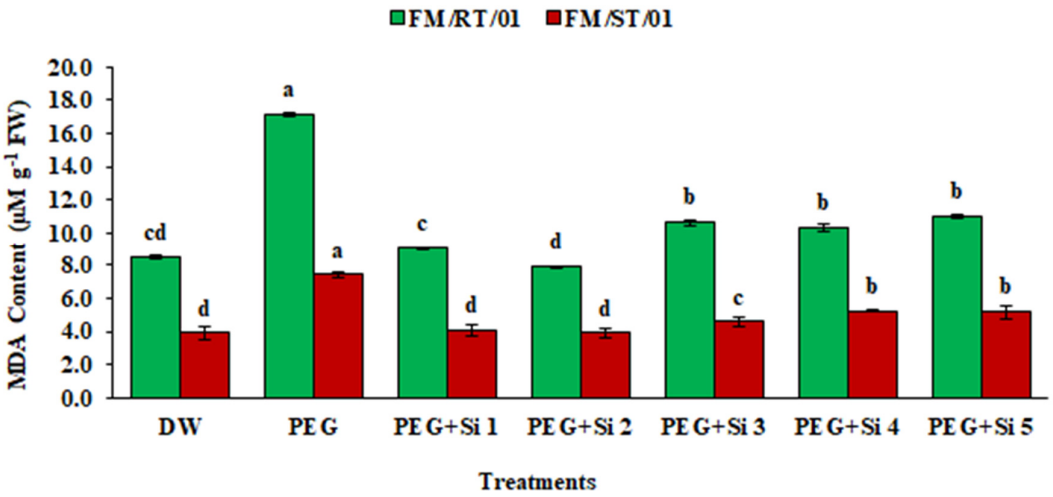

Figure 1. Effect of Si on lipid peroxidation (in terms of MDA content) in seedlings of local finger millet landraces under PEG-6000 induced osmotic stress

Values represent mean±SE. Values for each landrace with different alphabets are significantly different at p=0.05 as per Duncan's Multiple Range Test. Si: Silicon; FM/RT/01: Osmotic stress sensitive landrace; FM/ST/01: Osmotic stress tolerant landrace; DW: Distilled water; PEG: Polyethylene glycol (15%); Si 1: Si 5 ppm; Si 2: Si 10 ppm; Si 3: Si 15 ppm; Si 4: Si 20 ppm and Si 5: Si 25 ppm.

As compared to control, PEG-6000-induced osmotic stress resulted in accumulation of proline in the seedlings of both landraces as well as stressed seedlings of both landraces which received 5 and 10 ppm Si supplement. There was 4.62-fold and 3.92-fold increase in proline content in the seedlings of stress-tolerant and stress-sensitive landraces respectively. Supplementation of more than 10 ppm Si reduced proline accumulation in stressed seedlings. However, these proline levels were greater than those observed in the seedlings of both landraces not exposed to osmotic stress and Si supplementation (Figure 2A).

Glycine betaine content was significantly higher in the seedlings of finger millet grown in PEG (15%) environment as compared to control. Supplementation of 5 and 10 ppm Si reduced glycine betaine levels by 1.32-fold and 1.21-fold respectively in landrace (FM/ST/01). However, higher Si concentrations caused increase in the glycine betaine levels in the seedlings of FM/ST/01 landrace (Figure 2B).

Total soluble sugar content was higher in the seedlings of both the landraces grown under osmotic stress. Supplementation of 5 and 10 ppm Si further elevated these levels. Increase in Si concentration beyond 10 ppm resulted in reduced TSS content (Figure 2C). About 3.17-fold increase in TSS content was observed in PEG + 10 ppm Si treatment in osmotic stress-tolerant (FM/ST/01) landrace, whereas the increase was 2.73-fold in stress-sensitive landrace (FM/RT/01). The tendency of accumulation of osmolytes (proline and TSS) was more in osmotic stress-tolerant (FM/ST/01) landrace as compared to sensitive (FM/RT/01) landrace (Figure 2A, and C).

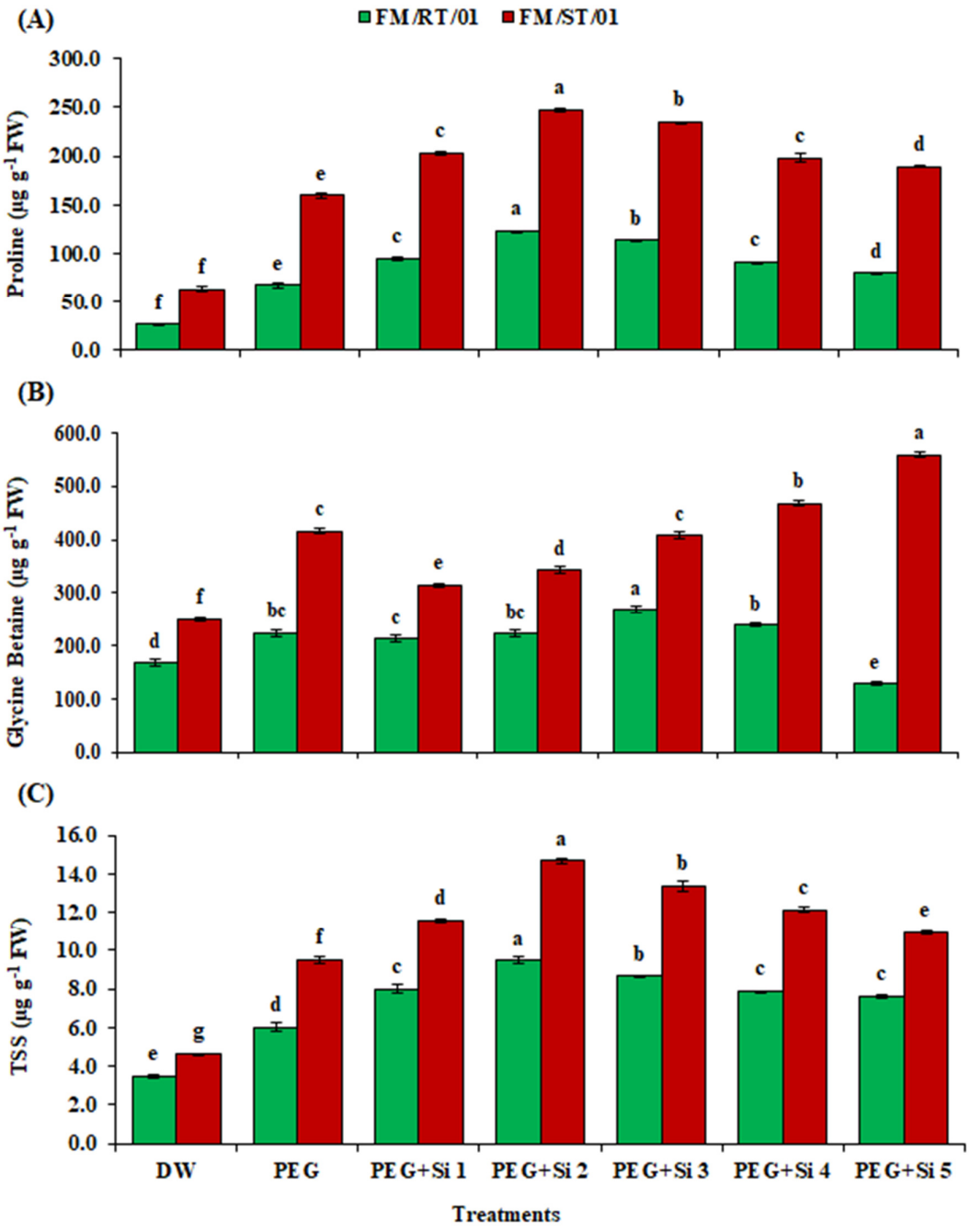

Figure 2 Effect of Si on osmolyte accumulation in seedlings of local finger millet landraces under PEG-6000 induced osmotic stress

A: Proline; B: Glycine Betaine; C: Total Soluble Sugars. Each value represents mean±SE. Values for each landrace with different alphabets are significantly different at p=0.05 as per Duncan's Multiple Range Test. Si: Silicon; FM/RT/01: Osmotic stress sensitive landrace; FM/ST/01: Osmotic stress tolerant landrace; DW: Distilled water; PEG: Polyethylene glycol (15%); Si 1: Si 5 ppm; Si 2: Si 10 ppm; Si 3: Si 15 ppm; Si 4: Si 20 ppm and Si 5: Si 25 ppm; TSS: Total soluble sugars.

The SOD levels were significantly up regulated in the seedling of FM/ST/01 grown under osmotic stress as compared to control. Supplementation of 5 ppm and 10 ppm Si to stressed seedlings of FM/ST/01 resulted in down regulation of SOD levels as compared to stressed seedlings However, with increase in the concentration of Si beyond 10 ppm, a gradual increase in SOD activity was observed (Figure 3A). On the contrary, the SOD was upregulated with increasing Si concentration up to 10 ppm and down regulated thereafter in sensitive landrace FM/RT/01 (Figure 3A). The activities of CAT, APX and GPX were up regulated with increasing concentration of Si up to 10 ppm as compared to control and PEG-induced stressed seedlings of both the landraces, but were downregulated at higher Si supplements (Figure 3B, C, and D). The up regulation of SOD, CAT, APX, and GPX was significantly higher in the seedlings of FM/ST/01 than those of FM/RT/01 (Figure 3).

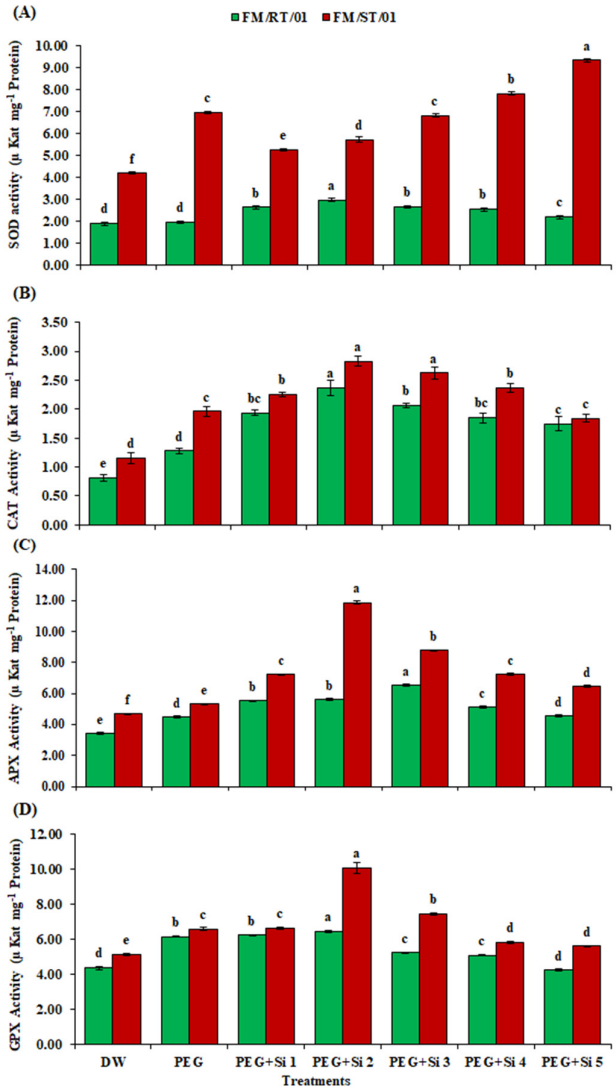

Figure 3 Effect of Si on antioxidant enzyme activities in seedlings of local finger millet landraces under PEG-6000 induced osmotic stress

A: SOD; B: CAT; C: APX; D: GPX. Each value represents mean±SE. Values for each landrace with different alphabets are significantly different at p=0.05 as per Duncan's Multiple Range Test. Si: Silicon; FM/RT/01: Osmotic stress sensitive landrace; FM/ST/01: Osmotic stress tolerant landrace; DW: Distilled water; PEG: Polyethylene glycol (15%); Si 1: Si 5 ppm; Si 2: Si 10 ppm; Si 3: Si 15 ppm; Si 4: Si 20 ppm and Si 5: Si 25 ppm.

Discussion

Successful seed germination and formation of healthy seedlings are significant stages in the field establishment of seed propagated plants. An understanding of how plants respond to various abiotic stresses during seed germination is important for elucidating the mechanisms of stress tolerance, survival of plants, and effective crop production (Almansouri *et al*., 2001; Ahire and Nikam, 2011). Drought stress is known to limit the seed germination as well as seedling development (Rizwan *et al*., 2015). In the present investigation, detrimental effects of PEG-induced osmotic stress culminated in reduced seed germination and weakened the seedlings of osmotic stress resistant as well as tolerant landraces (Tables 1 and 2). PEG-6000 is known to reduce the water potential around the seed which contributes to reduced germination percentage (Biju *et al*., 2017). The present investigation has clearly demonstrated that incorporation of 10 ppm Si in the PEG-environment can mitigate osmotic stress and improve the seed germination and seedling growth (Table 1). These results are consistent with sodium silicate primed alleviated seed germination under drought stress in wheat (Hameed *et al*., 2013). Likewise, silicon supplementation under osmotic stress was shown to improve the seed germination and seedling growth in tomato (Shi *et al*., 2014), durum wheat (Afef *et al*., 2016) and lentil (Biju *et al*., 2017). Since Si is hydrophilic in nature (Biju *et al*., 2017), its exogenous supplementation might have increased the water potential around seed thereby improving seed germination.

Generation of reactive oxygen species (ROS) is a typical response under osmotic stress. ROS cause peroxidation of membrane lipids which affects membrane fluidity and selectivity (Gossett *et al*., 1994). We observed relatively less damage to the lipid membranes in the stress-tolerant landrace FM/ST/01 as compared to stress-sensitive FM/RT/01 landrace (Figure 1) exposed to osmotic stress. Supplementation of silicon significantly lowered the MDA content in both the landraces suggesting its protective role against oxidative damage induced by osmotic stress. Addition of Si has been shown to inhibit overproduction of ROS thereby minimizing the peroxidation of membrane lipids to maintain the membrane integrity (Shi *et al*., 2016). Changes in lipid profile disrupts membrane composition and organization under stress conditions whereas cellular homeostasis leading to adjustment of lipid profiles offers tolerance to stress. In the present study, supplementation of Si might have helped to adjust the lipid profiles of the membranes thereby minimizing their damage. These results corroborate earlier results on improved stability of membrane lipids due to Si supplementation in rice (Agarie *et al*., 1998), chickpea (Gunes *et al*., 2007), and lentil (Biju *et al*., 2017) under drought stress.

Plants synthesize organic osmolytes in different capabilities and store them to tolerate the damage caused by osmotic stress (Ahire *et al*., 2013). Accumulation of osmolytes has been linked with a plant's capability to survive under different environmental stresses (Errabii *et al*., 2007; Slama *et al*., 2008). These osmolytes include complex carbohydrates, sugar alcohols, quaternary ammonium compounds such as glycine betaine, and an amino acid proline. These metabolites accumulate, apparently to defend cellular functions, at higher levels in plants suffering from low water potential (Saud *et al*., 2014). In our study, increased production of osmolytes was observed in both the landraces due to osmotic stress and simultaneous incorporation of Si as compared with control (Figures 2A and C). Si application might have activated the pathways of proline and sugar metabolism resulting in their accumulation. Our results corroborate with the observed increase in soluble sugars in sorghum leaves when treated with Si (Yin *et al*., 2014). Si supplementation to stressed seedlings of both the landraces resulted in a reduction in GB levels. The accumulation of GB was higher in stress-tolerant than stress-sensitive landrace (Figure 2B). Similar decline in GB levels was recorded in drought stressed lentil genotypes after supplementation of silicon (Biju *et al*., 2017). In the present study, osmolytes showed a varied response to the Si-mediated osmotic stress tolerance in finger millet which can be attributed to the varietal differences and diverse capabilities of genotypes to synthesize these osmolytes.

Abiotic stresses lead to oxidative stress through increase in ROS such as superoxide ($O2^{\bullet-}$) radicals, hydrogen peroxide (H_2O_2), and hydroxyl ($OH^{\bullet}$) radicals (Imlay, 2003). Plants have evolved an array of

protective mechanisms against these ROS. They include the production of low-molecular-mass antioxidants and antioxidative enzymes such as catalases, superoxide dismutases, peroxidases and glutathione reductases (Ahire *et al*., 2012). Among various antioxidative enzymes, SOD, CAT, and POD (peroxidases) make up the first line of defense in scavenging ROS. SOD converts superoxide radicals to H_2O_2, which is noxious to the nucleic acids, proteins, and chloroplast, and is dealt with by CAT and POD (Shen *et al*., 2010). In the present study, upregulation of SOD was observed in PEG treated seedlings as compared to the control (Figure 3A), whereas supplementation of Si (5 and 10 ppm) downregulated SOD in stressed seedlings. The decline in MDA content reveals marginal damage to membranes and downregulation of SOD upon supplementation of 10 ppm Si is suggestive of minimized production of toxic superoxide ($O2^{\bullet-}$) radicals.

As compared to control, silicon supplementation enhanced the activities CAT, APX and GPX in seedlings under osmotic stress conditions (Figure 3B, C, and D). Upregulation of these enzymes was observed in the osmotic stress-tolerant landrace (FM/ST/01) than the stress sensitive-landrace (FM/RT/01). The exogenous application of Si might have reduced the generation of superoxide ($O2^{\bullet-}$) radicals and PEG-induced osmotic stress might have generated relatively less toxic ROS like H_2O_2. APX plays an important role in scavenging H_2O_2 and CAT removes H_2O_2 by breaking it down to water and molecular oxygen. This can explain the higher levels of CAT, APX and GPX as compared to SOD. In summary, supplementation of Si has definitely triggered antioxidative defense machinery in both the landraces. Supplementation of Si to plants under stress has been shown to induces the antioxidative enzyme activities and reduce the production of ROS (de Oliveira *et al*., 2019). Among the different concentrations of Si used, supplementation of 10 ppm Si was found to be optimum to mitigate the PEG-6000-induced osmotic stress in finger millet.

Conclusions

The present study clearly demonstrates the potential of exogenous application of Si in enhancing seed germination, seedling growth and in mitigating oxidative stress in finger millet under PEG-induced osmotic stress via upregulating antioxidative defense mechanism. Therefore, an application of silicon fertilizer at the time of sowing would help in better stand establishment of finger millet.

Authors' Contributions

MLA, VTB and TDN have designed and supervised the research work and written the manuscript. PSM, MMS, SSS, SDU and MLA contributed to lab experiments, data collection, and analysis. SAK, PS and RBB has helped in designing experiments, in manuscript writing and editing.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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